

B/O Form PTO-1390		<b>Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 USC 371</b>		Attorney's Docket Number KIMB3006/REF	
				U.S. Application Number (if known) <b>10/030153</b>	
International Application Number PCT/KR00/00228		International Filing Date March 17, 2000		Priority Date Claimed July 7, 1999	
Title of Invention <b>A BIOFUEL CELL USING WASTEWATER AND ACTIVE SLUDGE FOR WASTEWATER TREATMENT</b>					
Applicant(s) for DO/EO/US <b>KIM et al.</b>					

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under 35 USC 371:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 USC 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.
  3. ☒ This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).
  4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
  5. ☒ A copy of the International Application as filed 35 USC 371(c)(2).
    - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☒ has been transmitted by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  - ☐ A translation of the International Application into English (35 USC 371(c)(2)).
  - ☒ Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
    - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☐ have been transmitted by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☒ have not been made and will not be made.
  - ☐ A translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)).
  - ☒ An oath or declaration of the inventor(s) (35 USC 371(c)(4)). ( ☐ Executed ☐ Unexecuted )
  10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)).
- Items 11 to 16 below concern other document(s) or information included:*
11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☒ A **FIRST** preliminary amendment.
  14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
  15. ☐ A substitute specification.
  16. ☐ A change of power of attorney and/or address letter.
  17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and U.S.C. 1.821 - 1.825.
  18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
  19. ☐ A second copy of the English translation of the international application under 35 U.S.C. 154(d)(4)
  20. ☒ Other items or information: Petition to Revive Abandoned Application Under 37 CFR 1.137(b) and Fee of \$640.00

Application Number (if Known) <b>10/030153</b>		International Application Number <b>PCT/KR00/00228</b>		Attorney's Docket Number <b>29 JAN 2002</b> <b>KIMB3006/REF</b>	
				Calculations	PTO USE ONLY
1. The following fees are submitted: <b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> <input checked="" type="checkbox"/> Neither International Preliminary Examination Fee (37 CFR 1.482) nor International Search Fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1040.00 <input type="checkbox"/> Search report has been prepared by the EPO or JPO ..... \$890.00 <input type="checkbox"/> International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) ..... \$710.00 <input type="checkbox"/> No International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) but International Search Fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$740.00 <input type="checkbox"/> International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00				\$1,040.00	
<b>ENTER APPROPRIATE BASIC FEE AMOUNT</b>				\$ <b>1,040.00</b>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
<b>CLAIMS</b>	<b>NUMBER FILED</b>	<b>NUMBER EXTRA</b>	<b>RATE</b>		
Total Claims	4 -20 =	0	× \$18.00	\$ 0.00	
Independent Claims	1 -3 =	0	× \$84.00	\$ 0.00	
Multiple Dependent Claims (if applicable)			+ \$280.00		
<b>TOTAL OF ABOVE CALCULATIONS</b>				\$ <b>1,040.00</b>	
Reduction by ½ for filing by small entity, if applicable. Small Entity Status is asserted pursuant to 37 CFR 1.27 for this application.				\$ 520.00	
<b>SUBTOTAL</b>				\$ <b>520.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).					
<b>TOTAL NATIONAL FEE</b>					
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property.				\$ 40.00	
<b>TOTAL FEES ENCLOSED</b>				\$ <b>560.00</b>	
				Amount to be:	
				Refunded:	
				Charged:	

- a. ☒ A check in the amount of \$1,200.00 (including Petition to Revive Fee of \$640.00) to cover the fees is enclosed.
- b. ☐ Please charge my **Deposit Account Number 02-0200** in the amount of \$\_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to **Deposit Account Number 02-0200**. A duplicate copy of this sheet is enclosed.

Note: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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 PATENT TRADEMARK OFFICE

Respectfully submitted,

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Attorney for Applicant: Richard E. Fichter  
 Registration Number: 26,382

DATE: January 28, 2002

PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

KIM et al.

U.S. National Phase of PCT/KR00/00228

Entry papers filed herewith January 29, 2001

For: A BIOFUEL CELL USING WASTEWATER AND ACTIVE SLUDGE  
FOR WASTEWATER TREATMENT

Attention: PCT OFFICE

**PRELIMINARY AMENDMENT  
AND INFORMATION DISCLOSURE STATEMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows:

**IN THE SPECIFICATION:**

Please add the following as the first paragraph of the application.

The present application is the U.S. national phase of international application number PCT/KR00/00228, filed March 17, 2000, which was published in English.

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A BIOFUEL CELL USING WASTEWATER AND ACTIVE SLUDGE FOR  
WASTEWATER TREATMENT

Technical Field

5       The present invention relates to a biofuel cell using  
wastewater as a fuel. More particularly, the present  
invention relates to a biofuel cell using organic  
substances contained in wastewater as a fuel, which biofuel  
cell can treat organism-containing wastewater while  
10       producing electricity. The biofuel cell according to the  
present invention allows reducing power generated from the  
catabolism of organic substances contained in wastewater by  
a microorganism to be converted directly into electrical  
energy.

15       Background Art

20       A biofuel cell is a device in which an organism or  
its part is used and by which reducing power generated from  
the energy metabolism of the organism can be converted into  
electrical energy. In the case of a microbial fuel cell,  
in order to convert reducing power generated from the  
oxidation of a substrate by a microorganism serving as a  
catalyst into electrical energy, electrons generated from  
the energy metabolism of the microorganism should be  
25       transferred from the microorganism to an electrode.  
However, most of organisms including microorganisms are  
surrounded by a lipid membrane, a non-conductive material,  
at their cells. For this reason, direct electron exchange  
between the microorganism and the electrode cannot be  
30       effected. Therefore, when a microorganism biomass is used  
as the catalyst, a suitable electron transfer mediator  
should be used to facilitate electron exchange between the  
microorganism and the electrode. As the electron transfer  
mediator, an electron carrier has been used that shows a  
35       strong lipophilic property in both the oxidized form and  
the reduced form, and is thus capable of passing through  
the membrane.

40       In particular, Roller et al. have proposed the use of  
*Proteus vulgaris*, *Escherichia coli*, *Atcaligenes eutrophus*,  
*Azotobacter chroococum*, or *Bacillus subtilis*, etc. as a  
catalyst, and thionine, methylene blue, brilliant cresyl

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blue, or benzyl viologen, etc. as an electron transfer mediator, in the biofuel cell (see, Roller et al., 1984, Journal of Chemical Technology and Biotechnology 34B: 3-12). According to Roller et al., an efficiency of the biofuel cell is significantly varied depending on the kind of the bacteria and the kind of the electron transfer mediator when being compared in view of the oxygen consumption amount.

Moreover, Bennetto et al. have disclosed a fuel cell using sugar as a fuel, a bacterium of a *Proteus* genus as a catalyst, and thionine as an electron transfer mediator. The disclosed fuel cell was reported to generate up to 44 coulombs (C) of electric current (see, Bennetto et al., 1985, Biotechnology Letters, 7:699-704). Further, Robin et al. have disclosed a biofuel cell using *Proteus vulgaris* as a biocatalyst, 2-hydroxy-1,4-naphtoquinone (HNQ) as an electron transfer mediator, and glucose as a fuel. The biofuel cell according to Robin et al. has an electromotive power of 0.5 milliamperes (mA) and 0.7 volts (V) (see, Robin et al., 1993, Applied Biochemistry and Biotechnology 39/40:27-40). In addition, according to Habermann and Pommer, there was reported a biofuel cell that utilizes cobalt oxide or molybdenum/vanadium alloy, etc. as an electrode, and hydrogen sulfide, as a fuel, produced by sulfate-reducing bacteria contained in wastewater, and that produces 150 mA/cm<sup>2</sup> of electric current (see, Habermann and Pommer, 1991, Applied Microbiology and Biotechnology 33:128-133).

Recently, there was separated anaerobic bacteria employing ferric ion, tetravalent manganese, hexavalent uranium, or hexavalent molybdenum, etc., as an electron acceptor. Substances, that can be used as a substrate for such metal salt-reducing bacteria, include aliphatic compounds, such as lactic acid, pyruvic acid, acetic acid, propionic acid, valeric acid, and alcohol, etc., and aromatic compounds, such as toluene, phenol, cresol, benzoic acid, benzyl alcohol, and benzaldehyde, etc. (see, Lovley and Klug, 1990, Applied and Environmental Microbiology 556: 1858-1864). Anaerobic bacteria are classified into fermentative bacteria and respiratory bacteria depending on their energy metabolism property.

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Fermentative bacteria decompose sugar and protein, etc. into organic acid, whereas respiratory bacteria completely oxidize fermentative products by the reduction of a suitable electron acceptor. Electron acceptors that can be used in the oxidation of organic substances by anaerobic respiratory bacteria include ferric oxide [Fe(II)], nitrate, manganese dioxide, sulfate, carbonate and the like. The reduction of ferric oxide among these electron acceptors is known to generate the largest energy by a reducing power generated from the oxidation of a given electron donor, with the energy level being low in order of nitrate, sulfate and carbonate (see, Byoung-Hong, Kim, Microorganism Physiology, Academy Press Co., Ltd., Seoul, Korea, 1995).

It is known that, where the iron-reducing bacteria are cultured in an anaerobic condition because of very low solubility of a ferric compound as an electron acceptor in water, about 65% of their cytochromes are arranged on their outer cell membrane. By such cytochrome arrangement, reducing power generated by the oxidation of organic substances within their cells is transferred outside of the cell to reduce ferric ion outside of their cells (see, Mayers and Mayers, Journal of Bacteriology 174: 3429-3478, 1992). Moreover, it was reported that *Shewanella putrefaciens* IR-1, an iron-reducing bacterium, can generate electric current without an electron transfer mediator, by being supplied with lactic acid or hydrogen, as an electron donor (see, Park et al., 1996, Abstract, I&EC Special Symp., Sept., 16-19).

Meanwhile, since wastewater introduced into a waste water disposal plant can contain iron at a high concentration and also ferric hydroxide is used as a phosphorus-removing agent, there will be present iron at a relatively high concentration in the wastewater disposal plant (see, Ledecke et al, 1989, Water Science and Technology 21: 325-337,). Thus, the ferric-reducing bacteria were reported to be present in most of active sludge in the wastewater disposal facility (see, Nielson et al, 1996, Water Science and Technology 34: 129-136). Also, it was reported that, in an anaerobic store condition of the active sludge, the reduction of ferric ion by

microorganisms contained in the active sludge has occurred and the iron-reducing bacteria were present at a significant amount (see, Rasmussen et al., 1994, Water Research 28: 417-425).

5       Based on the facts described above, where a variety of microorganisms present in active sludge or wastewater, etc. are anaerobically cultured in an anodic compartment, there will finally survive only microorganisms that are capable of employing, as an electron acceptor, an electrode  
10       having a given electric potential other than the components of the culture. As a result, using such a method, electrochemically active bacteria among a variety of microorganisms present in wastewater or active sludge can be selectively densely cultured, and the respective  
15       electrochemically active microorganisms can be isolated which are inherently present in various wastewaters.

#### Disclosure of the Invention

20       It is therefore an object of the present invention to provide a biofuel cell that is capable of purifying wastewater while producing electricity by carrying out an efficient electrode reaction using a variety of wastewaters and sludges without using an electron transfer mediator.

25       It is other object of the present invention to provide a method of treating wastewater while generating electric current by using an electrochemically active microorganism contained in wastewater and active sludge.

30       According to the present invention, the above objects can be accomplished by a biofuel cell comprising cathodic and anodic compartments defined in the interior of the biofuel cell and contained with conductive medium, respectively; an anode arranged in the anodic compartment ; a cathode arranged in the cathodic compartment ; and an ion exchange membrane interposed between the cathodic and  
35       anodic compartments and serving to divide the anodic compartment from the cathodic compartment , wherein the anodic compartment contains wastewater and active sludge.

40       As described above, among microorganisms present in wastewater and active sludge contained in a biofuel cell according to the present invention, electrochemically active species grow using an electrode of a certain

electric potential as an electron acceptor, thereby being densely cultured. Thus, the biofuel according to the present invention is operated using the densely cultured microorganisms, as a catalyst, and organic substances present in wastewater, as a fuel.

#### Brief Description of the Drawings

The above and other objects and aspects of the invention will be apparent from the following description of embodiments with reference to the accompanying drawings, in which:

Fig. 1 is a schematical view showing a biofuel cell of the present invention comprising a cathode, an anode, and a cation exchange membrane serving to divide the electrodes from each other, in which graphite felts are used as the respective electrodes,

Fig. 2 is a graph showing a reduction in electric current, electricity quantity (coulomb), and COD, which results from the use of a starch wastewater and an aerobic sludge in a biofuel cell of the present invention,

Fig. 3 is a graph showing a reduction in electric current, electricity quantity (coulomb), and COD, which results from the use of a starch wastewater and an anaerobic sludge in a biofuel cell of the present invention,

Fig. 4 is a graph showing a reduction in electric current, electricity quantity (coulomb), and COD, which results from the use of a livestock wastewater and an anaerobic sludge in a biofuel cell of the present invention,

Fig. 5 is a graph showing a reduction in electric current, electricity quantity (coulomb), and COD, which results from the use of a wastewater from septic tank and an anaerobic sludge in a biofuel cell of the present invention,

Fig. 6a is a photograph taken with a scanning electron microscope for the surface of an electrode which is in a state before being used in a biofuel of the present invention, and

Fig. 6b is a photograph taken with a scanning electron microscope for electrochemically active



microorganisms attached onto the surface of an electrode which is in a state after being used in a biofuel cell.

#### Best Mode for Carrying Out the Invention

5 Fig. 1 is a schematical view showing the structure of a biofuel cell according to the present invention. As shown in Fig. 1, the biofuel cell includes a cathodic compartment 12 and an anodic compartment 14. The cathodic and anodic compartments 12 and 14 have an oxygen  
10 introducing port 16 and a nitrogen introducing port 18, respectively. Also, in the cathodic and anodic compartments, there are arranged a cathode 22 and an anode 24, respectively. For the cathode 22 and the anode 24 of the biofuel cell, there can be used a graphite felt, a kind  
15 of graphite electrode. Moreover, in order to minimize resistance of the biofuel cell itself, a cation exchange membrane 26 is interposed between the cathodic and anodic compartments 12 and 14. Further, in the cathodic and anodic compartments 12 and 14, conductive media for the respective  
20 electrodes 22 and 24 are included. As the conductive medium for the cathode 22, a buffer solution is used, with the preferred buffer solution being 50 mM of phosphate buffer solution adjusted to pH 7. The cathode compartment 12 is maintained at a saturated condition by being  
25 continuously introduced with air, while the anode is maintained at an anaerobic condition by being introduced with nitrogen from which oxygen was completely removed by a passage of nitrogen through a gas oven. Additionally, in Fig. 1, reference numerals 32 and 34 represent an  
30 electrometer and a resistance terminal, respectively.

By the anaerobic condition described above, among bacteria present in wastewater and active sludge, only microorganisms capable of using an electrode as an electron acceptor can finally survive. As a result, the  
35 electrochemically active bacteria can be selectively densely cultured. The densely cultured microorganism species are used as a microorganism catalyst in the biofuel cell, such that they catabolize a variety of organic substances present in wastewater. Reducing power generated  
40 from the catabolism of the organic substances is used in the reaction with the electrode, thereby allowing electric

power to be generated. Additionally, as the organic substances present in wastewater are catabolized with the densely cultured microorganisms, a concentration of the organic substances in wastewater are reduced, thereby allowing a wastewater treatment effect to be achieved.

It is preferable to use a starch wastewater and an anaerobic sludge in the anodic compartment 14 of the biofuel cell according to the present invention while using a starch wastewater and an aerobic sludge in the cathodic compartment 12. On the anodic compartment 14 that is maintained at the anaerobic condition, the densely cultured, electrochemically active bacteria produce electric current while using the organic substances in wastewater as a fuel. A cation generated from the anodic compartment 14 is passed through the cation exchange membrane 26 by which the anodic compartment 14 is divided from the cathodic compartment 12. After passing through the cation exchange membrane 26, the cation is sent to the cathodic compartment 12 saturated with oxygen, and is converted into water in the cathodic compartment 12, thereby allowing electric current to be continuously produced. At the same time, the organic substances present in wastewater in the cathodic compartment are catabolized with the aerobic microorganisms, such that COD of wastewater can be reduced. As a result, it is possible to treat wastewater on both the cathodic and anodic compartments 12 and 14, simultaneously.

The following examples are for further illustration purposes only and in no way limit the scope of this invention.

#### Example 1

In this example, microorganisms using iron as an electron acceptor among microorganisms present in wastewater contained in the biofuel cell of the present invention were measured for a change in their colony number. In this measurement, a phosphate buffer solution-based medium (PBBM) was used as a medium. The following components were added to the medium to prepare a plate medium: 1g/L of an yeast extract, 1g/L of ammonium chloride, 25 ml/L of Macro-mineral (II) (including, per 1L, 6 g of  $\text{KH}_2\text{PO}_4$ , 12 g of  $\text{NaCl}$ , 2.4 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 1.6g

of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), 2 ml/L of microelements (including 12.8 g of nitroacetic acid, 0.1 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.17 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.1 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.1 g of  $\text{ZnCl}_2$ , 0.02g of  $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ , 0.01 g of  $\text{H}_3\text{BO}_3$ , 0.01g of molybdate, 1.0 g of NaCl, 0.017 g of  $\text{Na}_2\text{SeO}_3$ , and 0.026 g of  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ), 0.1 ml/L of a vitamin solution (including 0.002 g of biotin, 0.002 g of folacin, 0.010 g of B6(pyridoxin)HCl, 0.005 g of B1(thiamin)HCl, 0.005 g of B2(riboflavin), 0.005 g of nicotinic acid(niacin), 0.005 g of panthothenic acid, 0.0001g of B12 (cyanocobalamine) crystal, 0.005 g of PABA, and 0.005 g of lipoic acid (thioctic acid)), 1ml/L of resazurin (0.2%), and 1.8% of agar.

As an electron donor, 20 mM of acetic acid, 30 mM of lactic acid, and 20 mM of glucose were used, while 20 mM of ferric pyrophosphate, a water soluble iron, was used as an electron acceptor. In the first time of measurement, the respective samples of the aerobic sludge and the anaerobic sludge of the biofuel cell at the early stage of reaction were diluted with a physiological saline solution (0.8% brine) and then measured for Colony Forming Unit per ml of solution. In the second and third times, measurements were carried out using the same medium and method as in the first time, at one month and two months after the reaction, respectively. Results are shown in Table 1 below.

Table 1: Change in Colony Number in Biofuel Cell

Sample	Electron donor (mM)	Electron acceptor (mM)	First time	Second time	Third time
Aerobic sludge	Acetic acid(20))	FP(20)	$2.8 \times 10^7$	$0.9 \times 10^4$	$5.1 \times 10^3$
	Glucose(20)	FP(20)	$8.0 \times 10^7$	$1.3 \times 10^5$	$4.2 \times 10^4$
	Lactic acid(30)	FP(30)	$6.4 \times 10^7$	$1.1 \times 10^5$	$4.1 \times 10^4$
Anaero-bic sludge	Acetic acid(20)	FP(20)	$3.6 \times 10^5$	$5.4 \times 10^5$	$1.5 \times 10^5$
	Glucose(20)	FP(20)	$2.1 \times 10^5$	$8.4 \times 10^5$	$1.4 \times 10^5$
	Lactic acid(30)	FP(20)	$1.7 \times 10^5$	$1.5 \times 10^5$	$2.3 \times 10^5$

FP: Ferric Pyrophosphate

As evident from Table 1 above, in the case of the aerobic sludge sample, it is believed that, as the anodic

compartment of the biofuel cell is maintained in an anaerobic condition, strains other than facultative anaerobic strains are continued to reduce while being screened, such that only electrochemically active microorganisms are densely cultured. In the case of the anaerobic sludge sample, the anaerobic bacteria were increased at the second time, and then decreased at the third time, such that only electrochemically active microorganisms were densely cultured.

#### Example 2

This example is to examine characteristics of a biofuel cell using a starch wastewater (collected from Samyang Genex, Co., Inchon, Korea) and an aerobic sludge (collected from Samyang Genex, Co., Inchon, Korea). For this purpose, 350 mg of a graphite felt was used for the respective electrodes of cathode and anode. As a conductive medium for the cathode, 50 mM of phosphate buffer solution was used, and the cathodic compartment and the anodic compartment were connected through a cation exchange membrane. The conductive medium for the cathodic compartment was continuously introduced with air such that it was maintained in a condition where it was saturated with oxygen. The anodic compartment was introduced with nitrogen from which oxygen has been completely removed by a passage of nitrogen through a gas-purifying oven. Thus, the anodic compartment was removed in dissolved oxygen such that it was maintained in an anaerobic environment. All buffer solutions used in the test were adjusted to pH 7.0. Resistance of the fuel cell was set to infinity at the early stage of the reaction. When voltage reached a maximum, electric current produced at a resistance of 1 k $\Omega$  was measured. A biofuel cell was used in which the aerobic sludge and the starch wastewater were mixed in the volume ratio of 1:4. The volume of the aerobic sludge and the starch wastewater contained in the biofuel cell was 25 ml in total. As electric current generated by the organic substances present in the starch wastewater was decreased, 5 ml of wastewater was added. The generated voltage was measured at an interval of 120 seconds with Potential Start Meter (2000 multimeter, Keithley Instrument, Inc., USA).

The measured voltage was divided by resistance ( $1k\Omega$ ) to be converted into electric current. Chemical oxygen demand (COD) of wastewater was analyzed using a standard method (see, Standard Method for the Examination of Water and Wastewater, Closed Reflux Method, 19th edition, 1995). As can be seen in Fig. 2, electric current was generated up to 0.21 mA, electricity quantity (coulomb) was increased up to 26.5 C, and COD was reduced from 1100 ppm to 58 ppm. From this experiment, it was confirmed that reducing power generated from the oxidation of a substrate in wastewater was consumed directly by an electrode to generate electric current, and also to purify the starch wastewater.

### Example 3

In this example, a biofuel cell using starch wastewater and anaerobic sludge (collected from Samyang Genex, Co., Ltd., Inchon, Korea) was tested for a electric current productivity and a wastewater treatment ability. In this test, the condition and analysis method for the biofuel cell was the same as described in Example 1.

A biofuel cell was used in which an anaerobic sludge and a starch wastewater were mixed in the volume ratio of 1:4. The volume of the anaerobic sludge and the starch wastewater contained in the biofuel cell was 25 ml in total. As can be seen Fig. 3, electric current was generated up to 0.22 mA, quantity of electricity was increased up to 26.7 Coulomb, and COD was reduced from 1940 ppm to 55 ppm. From this experiment, it was therefore confirmed that reducing power generated from the oxidation of a substrate present in starch wastewater was consumed directly by an electrode to generate electric current, and also to purify the starch waste water.

Meanwhile, in order to examine a cultured degree of microorganisms incubated on the electrode which was used in the biofuel cell of the present invention, the electrode was photographed at its surface with an electron microscope (S-4100, FE-SEM, Hitachi, Japan) before being used in the biofuel cell. Also, after using the electrode in the biofuel cell, the electrochemically active microorganisms attached onto the electrode surface were photographed with the electron microscope. The photographed results are

shown in Fig. 6a for the electrode surface and 6b for the electrochemically active microorganisms. As can be seen in Figs. 6a and 6b, it could be confirmed that the electrochemically active microorganisms were attached onto the surface of the electrode.

#### Example 4

In this example, a biofuel cell was tested for an electric productivity and a wastewater treatment ability according to the same method as described in Example 2, except that a livestock wastewater (collected from Ansan Livestock, Ansan, Korea) was used instead of the starch wastewater. Also, the condition and the analysis method for the biofuel cell were the same as described in Example 1. As can be seen in Fig. 4, electric current was generated up to 0.21 mA, quantity of electricity was increased up to 12 Coulombs, and COD was reduced from 1030 ppm to 350 ppm. From this experiment, it was therefore confirmed that reducing power generated from the oxidation of a substrate present in the livestock wastewater was consumed directly by an electrode to generate electric current, and also to purify the livestock wastewater.

#### Example 5

In this example, a biofuel cell using a wastewater from a septic tank (collected from Apt. in Korea Institute of Science and Technology, Seoul, Korea) was tested for an electric productivity and a wastewater treatment ability. The operating condition and the analysis method for the biofuel cell were equal to those in Example 1. As can be seen in Fig. 5, electric current was generated up to 0.05 mA, quantity of electricity was increased up to 2.3 Coulombs, and COD was reduced from 680 ppm to 250 ppm. From this experiment, it was therefore confirmed that reducing power generated from the oxidation of a substrate in the wastewater from a septic tank was transferred directly to the electrode to generate electric current, and also to purify the wastewater from a septic tank.

#### Industrial Applicability

As apparent from the above description, the present invention provides the biofuel cell utilizing wastewater and sludge. In this biofuel cell, a portion of reducing power generated when the electrochemically active microorganisms contained in the sludge are subjected to the energy metabolism with the substrate present in wastewater, is utilized for the production of a biomass. At the same time, the remaining portion of the reducing power is utilized to produce electric current while purifying wastewater. As a result, where the biofuel cell utilizes a variety of wastewaters as a fuel, it then can achieve the electrical energy production and the wastewater treatment effect, simultaneously.

Although the preferred embodiments of the invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

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## Claims

1. A biofuel cell comprising cathodic and anodic compartments defined in the interior of the biofuel cell and contained with conductive medium, respectively; an anode arranged in the anodic compartment; a cathode arranged in the cathodic compartment; and an ion exchange membrane interposed between the cathodic and anodic compartments and serving to divide the anodic compartment from the cathodic compartment, wherein the anodic compartment contains wastewater and active sludge and is maintained in an anaerobic condition during an operation of the biofuel cell.

2. The biofuel cell of Claim 1, in which the active sludge and the wastewater are selected from the group consisting of a starch wastewater, a livestock wastewater, a wastewater from a septic tank, and a combination thereof.

3. The biofuel cell of Claim 1, in which the anodic compartment contains the sludge and the wastewater.

4. A method of treating wastewater while producing electric power using the biofuel cell of Claim 1, comprising of:

introducing the wastewater and the active sludge into the anodic compartment of the biofuel cell;

introducing nitrogen into the anodic compartment to remove dissolved oxygen from the anodic compartment, such that the anodic compartment is maintained in an anaerobic condition,

continuously introducing air into the cathodic compartment, such that the cathodic compartment is maintained in a condition where it is saturated with oxygen, and

densely culturing electrochemically active microorganisms present in the wastewater and the active sludge,

whereby the cultured active bacteria are used as a microorganism catalyst, and organic substances present in the wastewater are used as a fuel.



## Abstract

The present invention provides a biofuel cell using wastewater as a fuel. Electrochemically active microorganisms present in wastewater and active sludge used in the present invention oxidize organic substances present in wastewater. Electrons generated from the oxidation are discharged outside of the microorganism cell and transferred directly to the electrode, thereby allowing electric current to be generated while allowing wastewater to be purified. The biofuel cell using the electrochemically active bacteria according to the present invention allows an electric energy of up to 0.22 mA to be generated, and also enables COD of the wastewater used as a fuel to be decreased from 1900 ppm to 55 ppm. Moreover, an efficiency of the biofuel cell is varied depending on the kind and concentration of wastewater.

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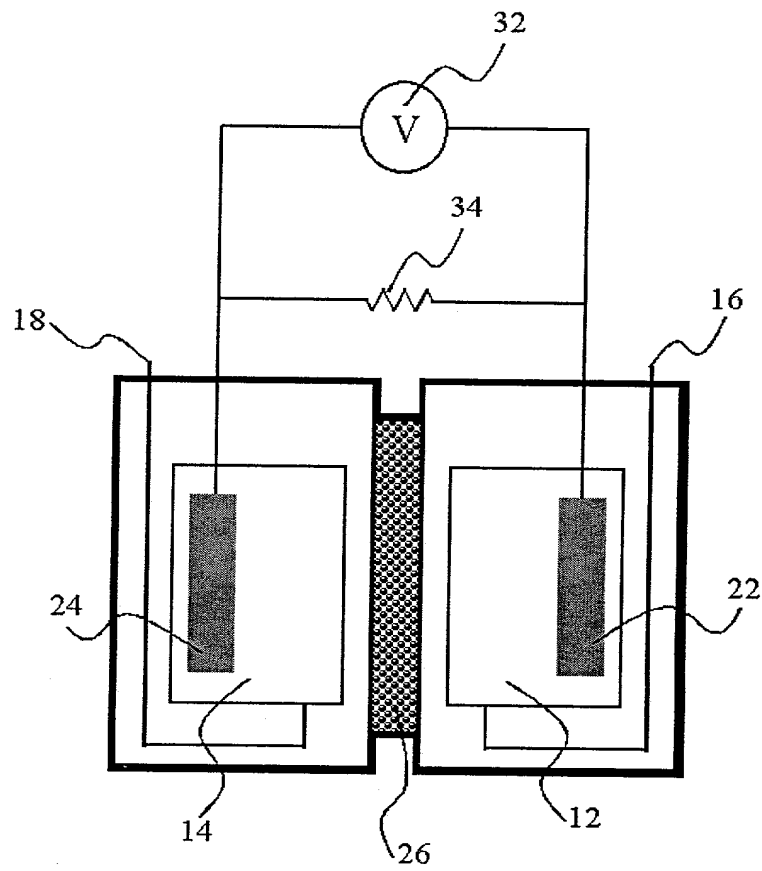
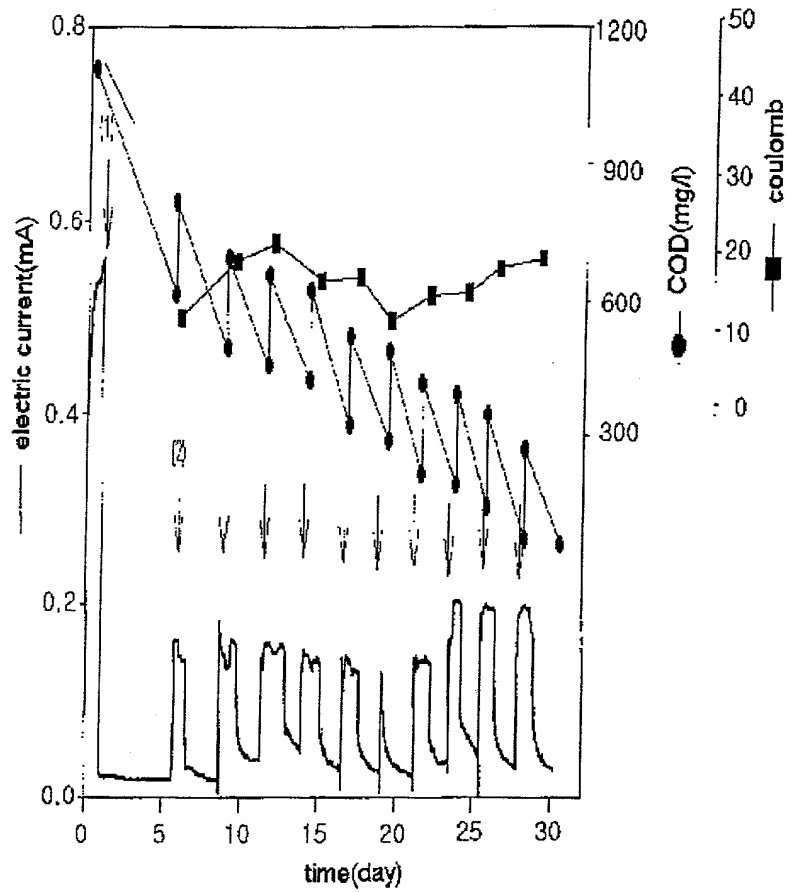
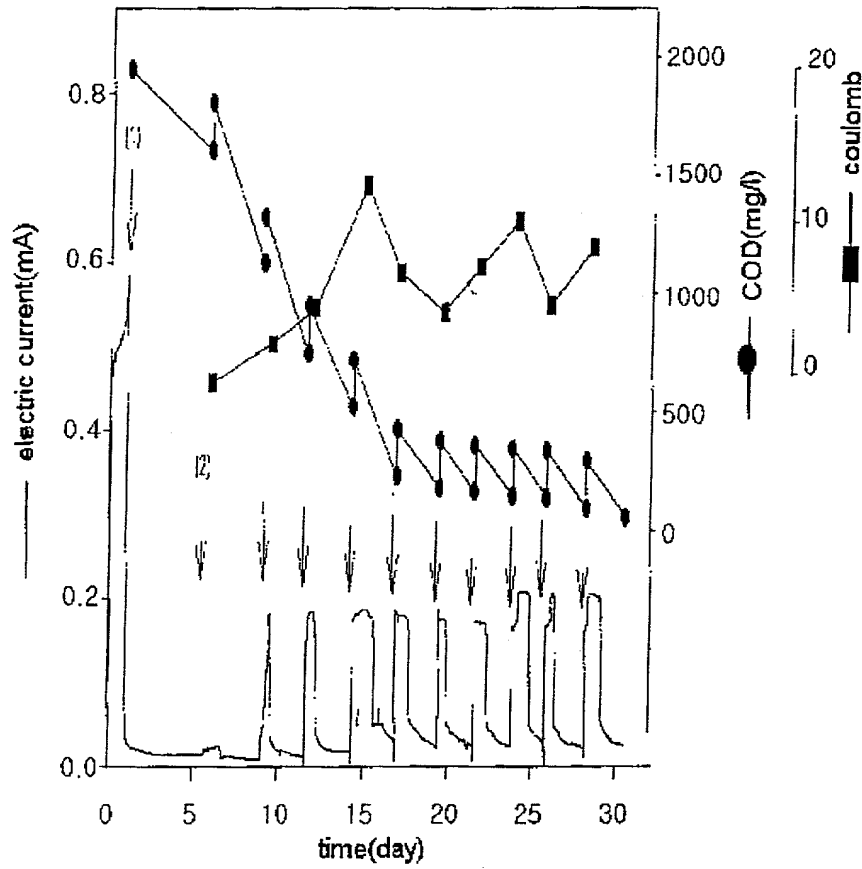


Fig. 1



(1)electric discharge (2) starch wastewater supply

Fig. 2.



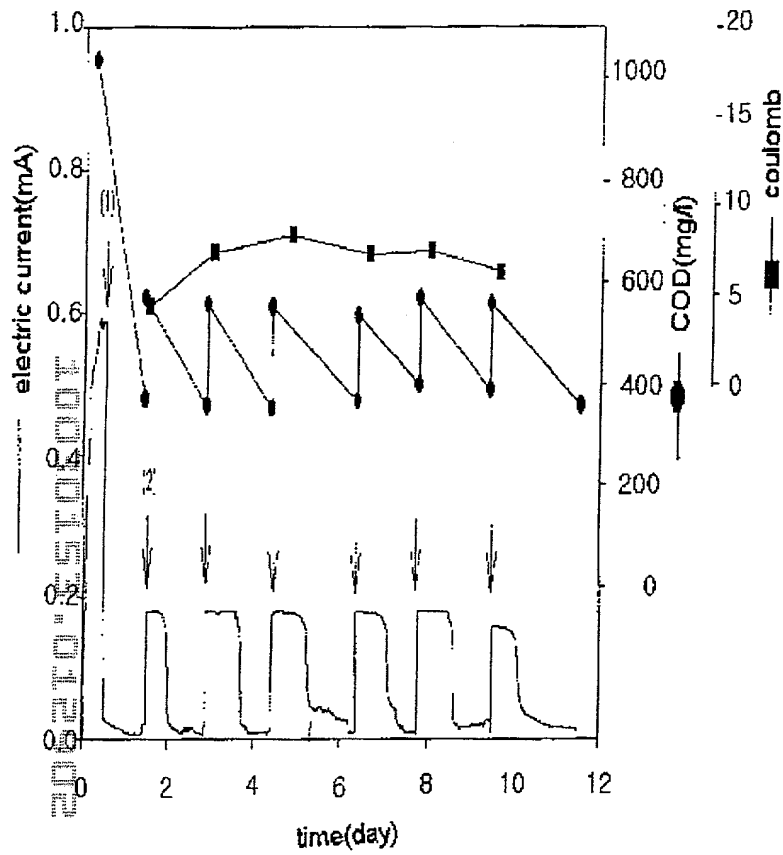
(1) electric discharge (2) starch wastewater supply

Fig. 3.

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(1) electric discharge (2) livestock wastewater supply

Fig. 4.

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**DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY**

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled:

**A BIOFUEL CELL USING WASTEWATER AND ACTIVE SLUDGE FOR WASTEWATER TREATMENT**

the specification of which (check one):

☐ is attached hereto, or ☒ was filed on: 17 March 2000

as U.S. Application Number or PCT International Application

Number: PCT/KR00/00228

and (if applicable) was amended on:

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56. I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			PRIORITY CLAIMED	
Number	Country	Day/Month/Year Filed	Yes	No
1999-27168	KOREA	07/07/99	X	

☐ Additional Priority Application(s) Listed on Following Page(s)

I HEREBY CLAIM THE BENEFIT UNDER TITLE 35 U.S. CODE §119(b) OF ANY U.S. PROVISIONAL APPLICATIONS LISTED BELOW.	
Application Number	Day/Month/Year Filed

☐ Additional Provisional Application(s) Listed on Following Page(s)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating The United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Number	Filing Date	Status - Patented, Pending or Abandoned
PCT/KR00/00228	17 March 2000	

☐ Additional US/PCT Priority Application(s) listed on following Page(s)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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(IN DOCUMENT FOR HS DECLARATION)

(DECLARATION)

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## CONTINUATION OF DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

Page 2

Prior Foreign Application(s) (35 USC §119)			Priority Claimed	
Number	Country	Day/Month/Year Filed	Yes	No

Prior Provisional Applications 35 U.S. Code §119(e)	
Application Number	Day/Month/Year Filed

Prior U.S. or PCT International Applications (35 U.S. Code §120)		
Application Number	Filing Date	Status - Patented, Pending or Abandoned

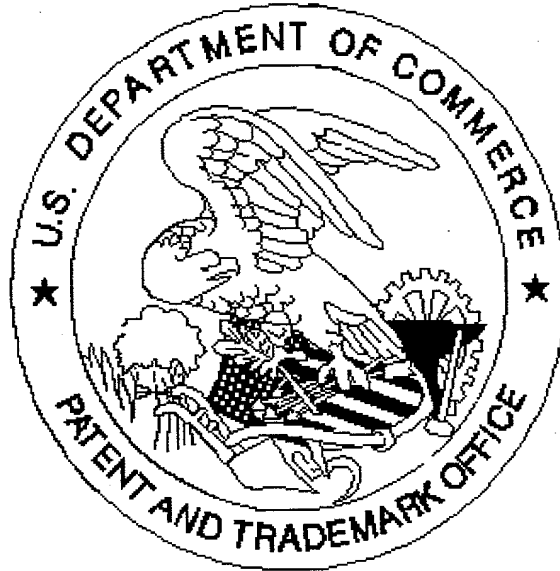
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